

Work continued from Page

Deprotection Prior to Cleavage & Deprotection

Synthesis of T-20mer final
DMTr- on in 24 mL Column
using standard position for
amide delivery

T amide = 2x10 gm bottles

Column Vol = 24

Dia-
meter = 35 mm

Loading of T-support = 94 $\mu\text{mol/gm}$

Synthesis Scale = $94 \times 7.5744 \text{ gm}$
= 712 μmol scale

weight of Column = 134.9257

+ support 142.5001

after Synthesis
was done = 148.1634

Total amount of support
+ oligo = 13.2377

① Regular deprotection at 60°C in
3 mL of NH_4OH for 16 hrs
amount = 0.1474 gms

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Work continued from Page

after 16 hrs. regular protocol

Total Vol = 10 mL

10 λ = 0.610 AU in 1 mL

Total AU = 610 in 10 mL of 0.1474 gm

Operator
Date
Sample No. 0012

54782.88 AU in 13.2377 gms

ABSORBANCE
260 nm 0.610 AU712 μ mole = 54782.88 AU1 μ mole = 76.94 AU/ μ molefor HPLC = 49.18 λ in 1 mL = 3 AU

② Amount for deprotection

at room temp = 0.1560 gm

after work up 10 λ = 0.948 AABSORBANCE
260 nm 0.000 AUOperator
Date
Sample No. 0010ABSORBANCE
260 nm 0.948 AU

10 mL = 948 AU / 0.1560

13.2377 gms = 80.4445 AU

112.98 AU/ μ mole

③ Amount for deprotection at 60°C with Imidazole

0.196 gms

10 mL Total, 10 λ = 1.205 AU = 1205 AU/0.196

81384.83 AU

114.3 AU/ μ moleOperator
Date
SIGN Sample No. 0011

CHICAGO 60605 MADE IN USA

Work continued to Page

DISC ABSORBANCE
260 nm 1.205 AU

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Work continued from Page

Expts for Decyanoethylation using
 2M TEA in dry CH_3CN

(1) amount of T-20 DmTr-ON

in 1ml column =

wt of column =

T-20 new =

after reaction =

Result: V3P6 wash 01

Method for Decyanoethylation

V3P6 wash 01

(Valve 3 position
 as it is not used
 in regular Syn.

Variables = Time = 10 minutes

Flow rate = 10 ml / minute

≈ 100 ml of
 2M TEA used

wt for deprotection =

0.1594 gms

at 60°C in 3ml NH_4OH for 16 hrs

10ml Total Volume

$10 \times = 1.967 \text{ AU/ml}$

IEX = 312 (3 AU)

Operator
 Date
 Sample No. 0014

ABSORBANCE
 260 nm 0.967 AU

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Expt: 2 Decyanoethylation with
2M TEA in dry CH_2CN over Sievers

Variable

Flow rate = 10 ml/minute

Time = 20 minutes

/ 200 ml
of
reagent
used

wt of Column = 25.6758

+ T-20mer = 26.3048

~~20mer~~ - Support + T-20mer = 0.6290

after reaction

Method V3P6 Wash

Result V3P6Wa02

weight for deprotection = V3P6Wa02 = 0.1582

Total Vol after work up 10 mL

10% = 1.026 AU in 1 mL

for IEX = 0.030 mL (3 AU)
30 μL Operator
Date 06 Jan 1999
Sample No. 0015ABSORBANCE
260 nm 1.026 AUAnula Bhau
Bla

Work continued from Page

Decyanoethylation of

LTP- 20 mer T- ON

Method

V3P6 Wash

Column

=

1 mL

2.0M Diethylamine
↑

V3P6 Wash

(2.0 M t-butylamine)
in ACN.

amount for deprotection at 60°C

for 16 hrs = 0.119 mg

Total Volume = 10 mL

10 A = 0.861 AU/mL

For IEX = 3.5 µL

chromatograms in the folder.

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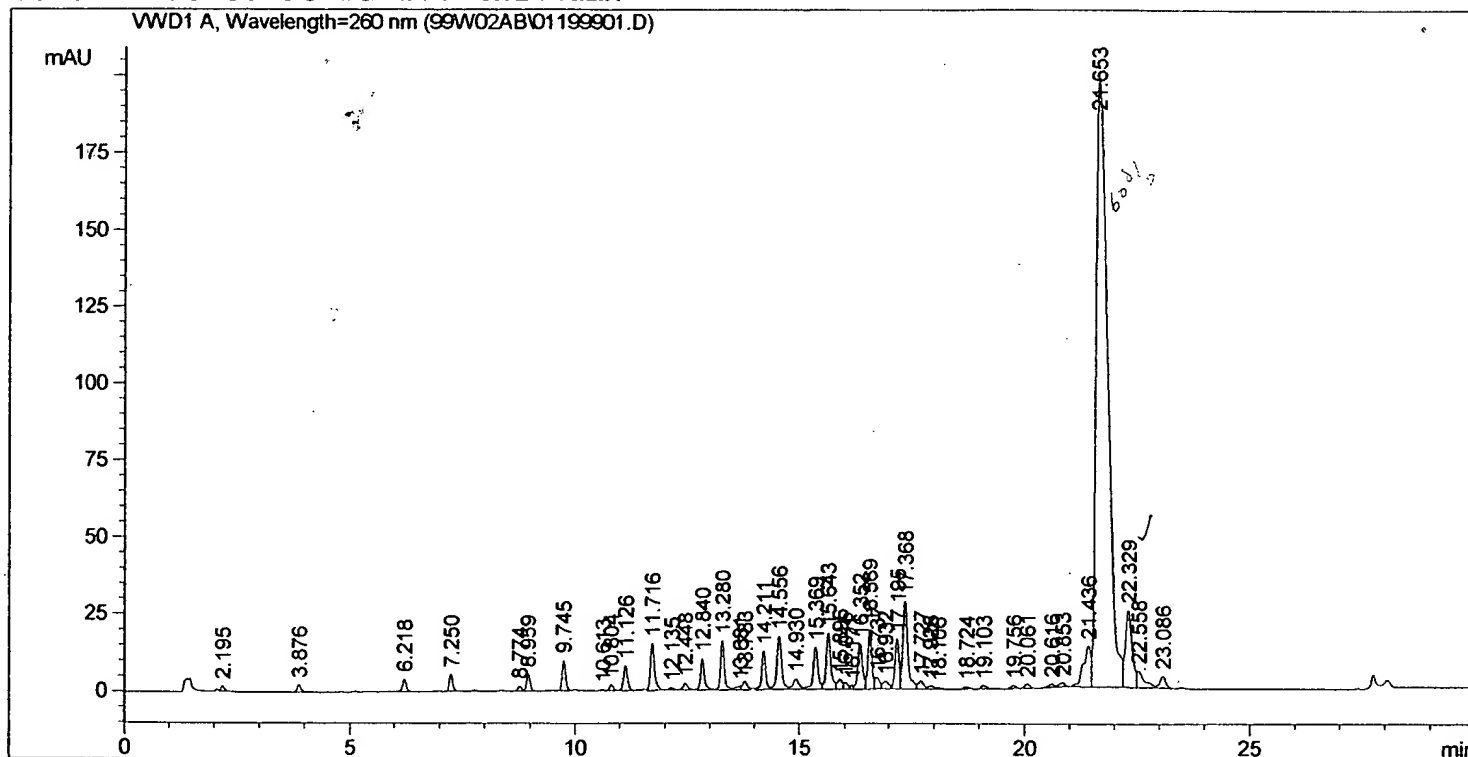
DATE

Sample: LJP 20 mer T- on, Ref. deprotection at 60 0 C.
 for 16 hrs. 3.0 AU / ml, 30 ul injection, NEW Column
 Method : KA 10_70
 Column : Nucleopac Pa -100, 4x250 mm, 1ml/min, temp +50C
 Buffer B: 10mM NaClO4, 1mM Tris pH9.3
 Buffer D: 1mM Tris +0. 3M NaClO4

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=====
Injection Date   : 1                               Seq. Line :    2
Sample Name     : LJP 20 T-on                      Vial       :    1
Acq. Operator   : Anila Bhan                       Inj        :    1
                                                    Inj Volume : 30 µl

Sequence File   : C:\HPCHEM\1\DATA\99W03AB\011999AB.S
Method          : C:\HPCHEM\1\METHODS\KA10_70.M
Last changed    :                               by Anila Bhan
Method for analysis of DNA oligos.
Column NucleoPac Pa-100 4x250mm.
  
```



Area Percent Report

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Sorted By      : Signal
Multiplier     : 1.0000
Dilution       : 1.0000
  
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LJP 20mer T-on (Normal Conditions)
 TG-20mer Deprotection NH₄OH @ 55°-60°C
 N+ = ~ 6.7%

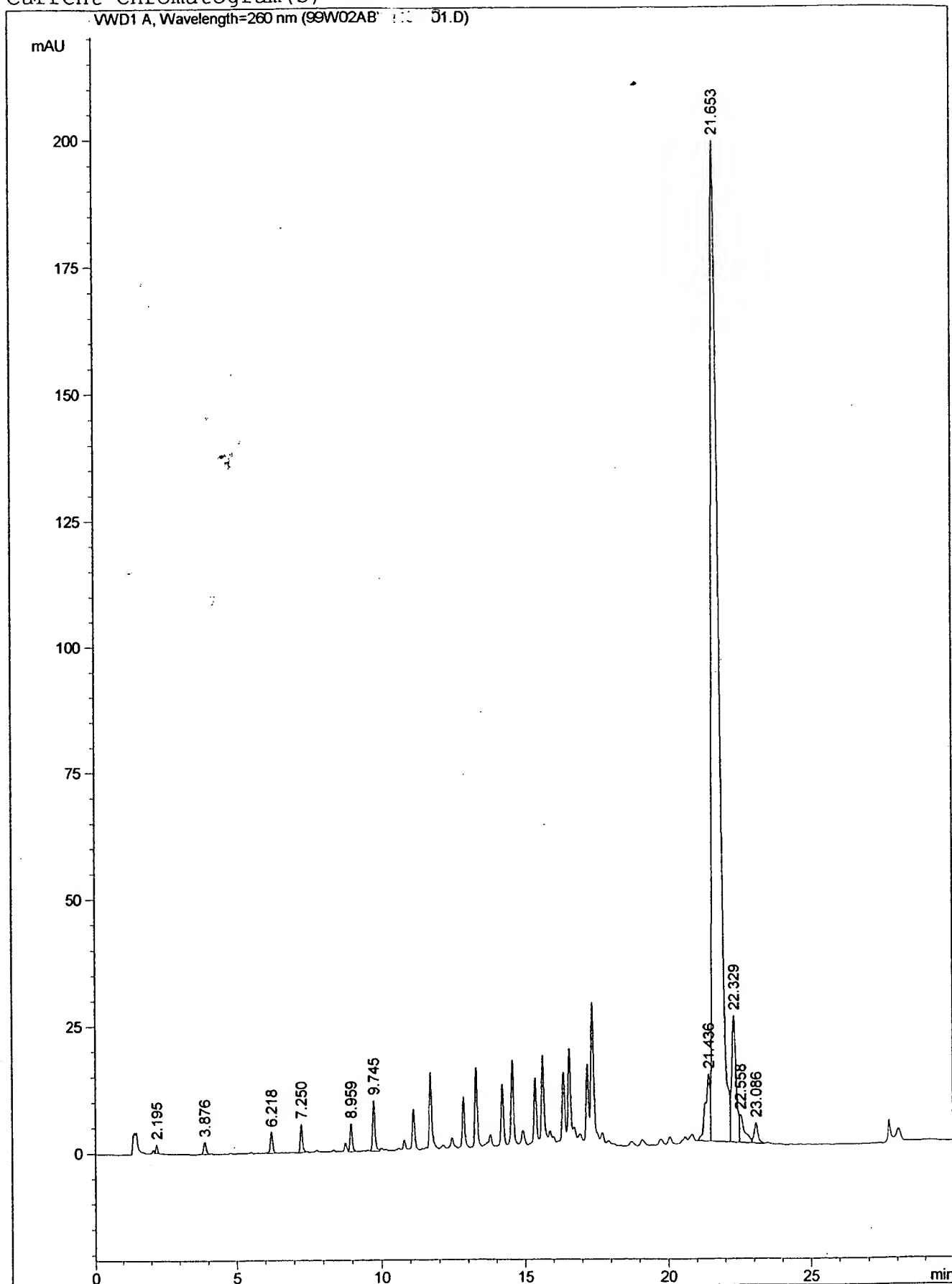
Signal 1: VWD1 A, Wavelength=260 nm
Results obtained with enhanced integrator!

Peak #	RetTime [min]	Type	Width [min]	Area mAU *s	Height [mAU]	Area %
1	2.195	VP	0.0756	9.25233	1.85617	0.1586
2	3.876	VV	0.0945	15.71768	2.47346	0.2694
3	6.218	VV	0.0861	24.51600	4.25996	0.4201
4	7.250	VV	0.0796	29.83348	5.73574	0.5113
5	8.774	VV	0.0814	9.60108	1.75170	0.1645
6	8.959	VV	0.0830	30.60193	5.56802	0.5244
7	9.745	VV	0.0843	56.25139	10.04331	0.9640
8	10.613	VV	0.1243	4.65430	5.22389e-1	0.0798
9	10.804	VV	0.0893	12.76557	2.11494	0.2188
10	11.126	VV	0.0905	48.99257	8.15273	0.8396
11	11.716	VV	0.0949	96.69289	15.43126	1.6570
12	12.135	VV	0.1298	8.33922	9.25929e-1	0.1429
13	12.448	VV	0.1208	20.67579	2.40194	0.3543
14	12.840	VV	0.0956	67.14149	10.40929	1.1506
15	13.280	VV	0.0952	101.45315	16.13787	1.7386
16	13.681	VV	0.1126	10.43183	1.24076	0.1788
17	13.783	VV	0.1026	20.50822	2.91086	0.3515
18	14.211	VV	0.0971	81.85365	12.69703	1.4027
19	14.556	VV	0.1043	123.39919	17.46878	2.1147
20	14.930	VV	0.1332	33.42327	3.54867	0.5728
21	15.369	VV	0.1081	100.87623	13.88523	1.7287
22	15.643	VV	0.1055	132.08507	18.41878	2.2636
23	15.896	VV	0.1037	23.77879	3.32964	0.4075
24	16.015	VV	0.0956	14.46146	2.20082	0.2478
25	16.171	VV	0.0795	6.80630	1.25158	0.1166
26	16.352	VV	0.1034	103.83770	14.85732	1.7795
27	16.569	VV	0.1045	133.04355	19.45940	2.2800
28	16.737	VV	0.1088	29.51185	3.96017	0.5057
29	16.932	VV	0.1371	24.74845	2.60270	0.4241
30	17.195	VV	0.0986	105.70080	16.38626	1.8114
31	17.368	VV	0.1134	217.66148	28.65527	3.7301
32	17.727	VV	0.1186	22.41374	2.78720	0.3841
33	17.926	VV	0.1374	10.72811	1.14092	0.1838
34	18.106	VV	0.1272	4.67473	5.39886e-1	0.0801
35	18.724	VV	0.1539	8.72009	9.11050e-1	0.1494
36	19.103	VV	0.1392	11.36009	1.22006	0.1947
37	19.756	VV	0.1349	10.46360	1.18692	0.1793
38	20.061	VP	0.1245	12.03598	1.49438	0.2063
39	20.616	VV	0.1288	10.55021	1.16672	0.1808
40	20.853	VBA	0.1369	13.69809	1.46269	0.2347
41	21.436	BV	0.1531	150.48209	13.37514	2.5788
42	21.653	VV	0.2967	3496.85034	197.66643	59.9259
43	22.329	VV	0.1659	282.17007	25.13445	4.8356
44	22.558	VV	0.1607	64.48777	5.53502	1.1051
45	23.086	VV	0.1381	38.03810	4.07063	0.6519

Totals : 5835.28969 508.34950

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Current Chromatogram(s)

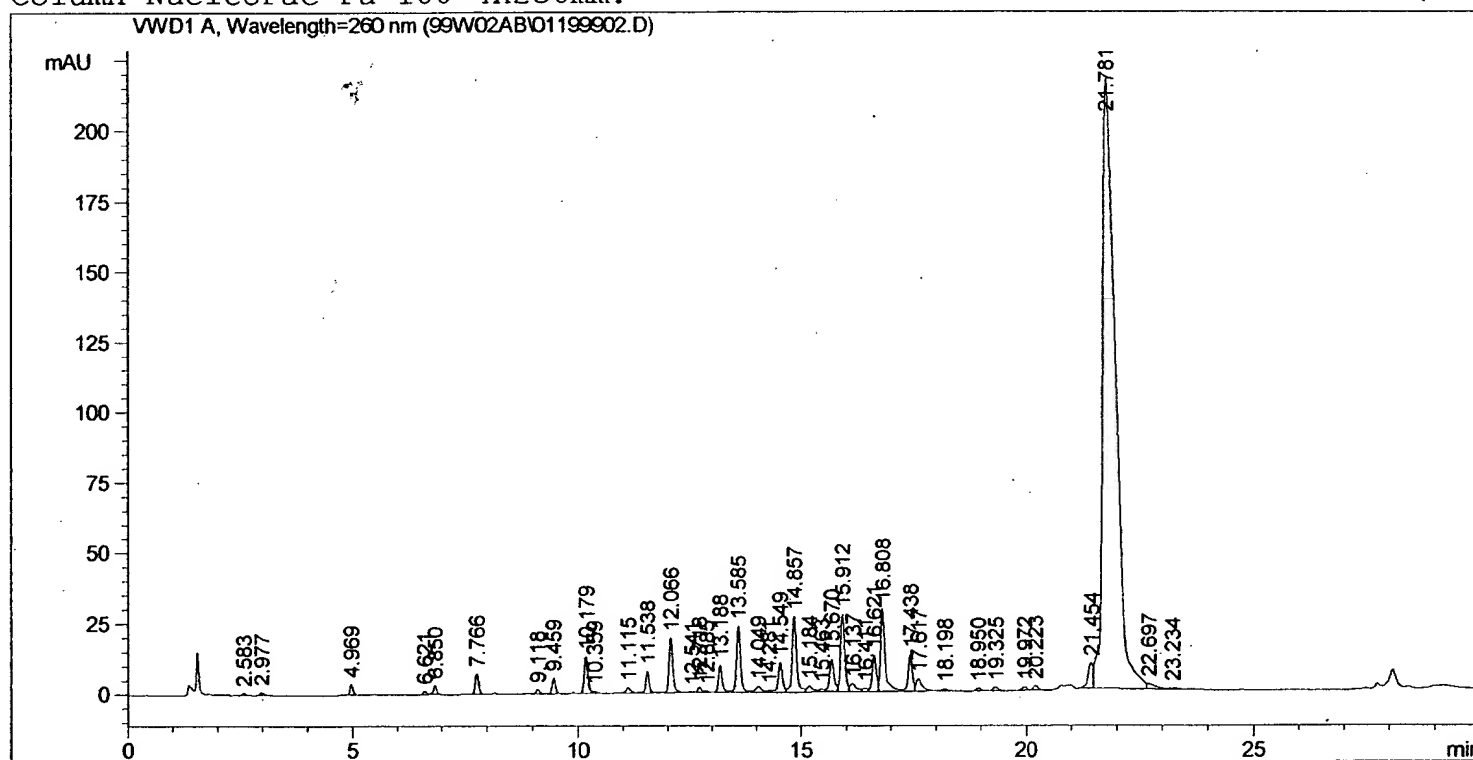


Sample: LJP 20 mer DMTr on, V3P6WA20 deprotection at
60 0 C.
for 16 hrs. 3.0 AU / ml, 30 ul injection, New Column
Method :KA 10_70
Column :Nucleopac Pa -100, 4x250 mm, 1ml/min, temp +50C
Buffer B: 10mM NaClO4, 1mM Tris pH9.3
Buffer D:1mM Tris +0. 3M NaClO4

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Injection Date	:		Seq. Line	:	5
Sample Name	:	LJP 20 T- on	Vial	:	2
Acq. Operator	:	Anila Bhan	Inj	:	1
			Inj Volume	:	30 µl

Sequence File : C:\HPCHEM\1\DATA\99W03AB\011999AB.S
Method : C:\HPCHEM\1\METHODS\KA10_70.M
Last changed : Anila Bhan
Method for analysis of DNA oligos.
Column NucleoPac Pa-100 4x250mm.



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Area Percent Report
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Sorted By	:	Signal
Multiplier	:	1.0000
Dilution	:	1.0000

LJP-20mer DMTr-ON, DEA washed prior to normal
DP at 60°C in NH₄OH (New Column)
NT = greatly reduced

Signal 1: VWD1 A, Wavelength=260 nm
Results obtained with enhanced integrator!

Peak #	RetTime [min]	Type	Width [min]	Area mAU	Height [mAU]	Area %
1	2.583	VV	0.0822	4.12494	7.60320e-1	0.0682
2	2.977	VP	0.1057	8.14528	1.13276	0.1347
3	4.969	VV	0.0760	20.10576	4.00523	0.3325
4	6.621	VV	0.0750	7.06265	1.39691	0.1168
5	6.850	VV	0.0774	17.25746	3.36130	0.2854
6	7.766	VV	0.0799	38.76381	7.41475	0.6410
7	9.118	VV	0.0985	11.04869	1.59102	0.1827
8	9.459	VV	0.0826	30.77767	5.63798	0.5089
9	10.179	VV	0.0822	71.68959	13.22168	1.1854
10	10.359	VV	0.0916	5.39034	8.48769e-1	0.0891
11	11.115	VV	0.1112	16.49422	2.08520	0.2727
12	11.538	VV	0.0881	46.06451	7.94184	0.7617
13	12.066	VV	0.0918	117.78107	19.63641	1.9476
14	12.541	VV	0.1634	5.38228	4.39118e-1	0.0890
15	12.718	VV	0.0969	13.94427	2.12721	0.2306
16	12.885	VV	0.0989	5.33004	7.91696e-1	0.0881
17	13.188	VV	0.0922	59.21531	9.81420	0.9792
18	13.585	VV	0.0959	149.38000	23.52205	2.4701
19	14.049	VV	0.1410	22.99424	2.25080	0.3802
20	14.281	VV	0.1210	6.51896	7.56208e-1	0.1078
21	14.549	VV	0.0977	68.57211	10.54562	1.1339
22	14.857	VV	0.1029	183.59241	26.90079	3.0358
23	15.184	VV	0.1306	21.77042	2.30381	0.3600
24	15.463	VV	0.1170	9.45533	1.17771	0.1564
25	15.670	VV	0.1014	78.57019	11.51672	1.2992
26	15.912	VV	0.1000	181.81581	27.63620	3.0065
27	16.137	VV	0.1346	29.95566	3.09850	0.4953
28	16.411	VV	0.1111	10.27938	1.32240	0.1700
29	16.621	VV	0.0988	83.71881	12.94446	1.3844
30	16.808	VV	0.1109	221.16414	29.46280	3.6571
31	17.438	VV	0.1037	90.26736	13.10271	1.4926
32	17.617	VV	0.1343	44.17767	4.58229	0.7305
33	18.198	VV	0.1730	11.97245	9.60820e-1	0.1980
34	18.950	VV	0.1314	10.21311	1.18158	0.1689
35	19.325	VV	0.1276	11.88926	1.40807	0.1966
36	19.972	VV	0.1304	11.87105	1.36785	0.1963
37	20.223	VV	0.1284	14.07700	1.67926	0.2328
38	21.454	BV	0.1072	66.45797	9.24104	1.0989
39	21.781	VV	0.3170	4201.24951	216.18849	69.4709
40	22.697	VV	0.1742	27.32028	2.11206	0.4518
41	23.234	VV	0.2846	11.63748	5.88382e-1	0.1924

Totals : 6047.49850 488.05701

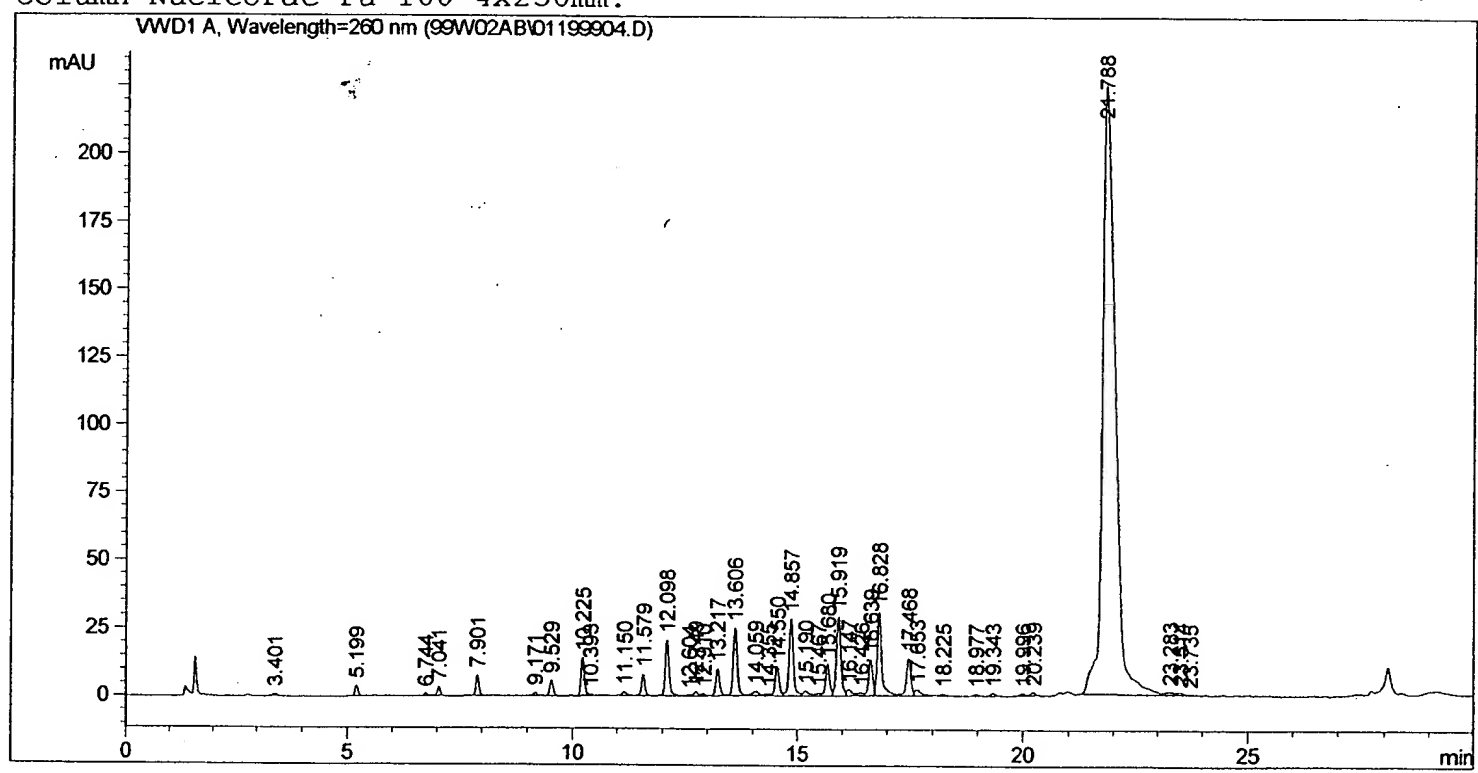
*** End of Report ***

Sample: LJP 20 mer DMTr on, V3P6WA20 deprotection
at ROOM TEMP. for 88 hrs. 3.0 AU / ml, 30 ul injection
New Column, ~~NucleoPac~~, DEA wash
Method :KA 10_70
Column :Nucleopac Pa -100, 4x250 mm, 1ml/min, temp +50C
Buffer B: 10mM NaClO4, 1mM Tris pH9.3
Buffer D:1mM Tris +0. 3M NaClO4

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Injection Date :	Seq. Line : 7
Sample Name : LJP 20 T- on	Vial : 4
Acq. Operator : Anila Bhan	Inj : 1
	Inj Volume : 30 µl

Sequence File : C:\HPCHEM\1\DATA\99W03AB\011999AB.S
Method : KA 10_70.M
Last changed : Anila Bhan
Method for analysis of DNA oligos.
Column NucleoPac Pa-100 4x250mm.



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Area Percent Report
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Sorted By : Signal
Multiplier : 1.0000
Dilution : 1.0000

LJP 20 mer DMTr- ON, DEA Washed prior to
DP at room temp. for 88 hrs. (New Column
N + Tailing but greatly reduced

Signal 1: VWD1 A, Wavelength=260 nm
Results obtained with enhanced integrator!

Peak #	RetTime [min]	Type	Width [min]	Area mAU *s	Height [mAU]	Area %
1	3.401	VV	0.1107	7.64108	1.05563	0.1195
2	5.199	VV	0.0782	20.36685	4.00571	0.3185
3	6.744	VV	0.0787	6.11193	1.16413	0.0956
4	7.041	VV	0.0832	19.87513	3.52594	0.3108
5	7.901	VV	0.0797	40.91972	7.85518	0.6399
6	9.171	VV	0.0854	7.73776	1.32954	0.1210
7	9.529	VV	0.0821	31.90540	5.88887	0.4989
8	10.225	VV	0.0834	76.36335	14.13334	1.1941
9	10.393	VV	0.0872	4.68988	7.84756e-1	0.0733
10	11.150	VV	0.1101	12.63591	1.61608	0.1976
11	11.579	VV	0.0901	49.29993	8.25321	0.7709
12	12.098	VV	0.0917	124.19886	20.74452	1.9421
13	12.604	VV	0.1656	5.35896	4.34823e-1	0.0838
14	12.749	VV	0.0918	10.74855	1.72198	0.1681
15	12.910	VV	0.1024	6.35274	9.03132e-1	0.0993
16	13.217	VV	0.0894	60.29174	10.18605	0.9428
17	13.606	VV	0.0946	156.54074	25.11654	2.4478
18	14.059	VV	0.1396	19.52973	1.93462	0.3054
19	14.355	VV	0.1549	10.07477	8.64259e-1	0.1575
20	14.550	VV	0.0936	67.57163	10.98817	1.0566
21	14.857	VV	0.1020	190.71521	28.28390	2.9822
22	15.190	VV	0.1353	20.15853	2.04657	0.3152
23	15.467	VV	0.1069	7.56235	1.03716	0.1183
24	15.680	VV	0.1014	82.30637	12.06723	1.2870
25	15.919	VV	0.1016	192.18681	29.18440	3.0052
26	16.147	VV	0.1276	22.90222	2.52705	0.3581
27	16.426	VV	0.1441	13.55002	1.29342	0.2119
28	16.639	VV	0.0991	87.66231	13.49792	1.3708
29	16.828	VV	0.1101	229.89104	30.89173	3.5948
30	17.468	VV	0.1066	97.53876	13.91002	1.5252
31	17.653	VV	0.1517	27.39659	2.46204	0.4284
32	18.225	VV	0.1576	9.70731	8.61699e-1	0.1518
33	18.977	VV	0.1401	7.25416	7.72843e-1	0.1134
34	19.343	VV	0.1251	9.46124	1.13327	0.1479
35	19.996	VV	0.1319	8.52873	9.81736e-1	0.1334
36	20.239	VV	0.1294	10.83523	1.29864	0.1694
37	21.788	BV	0.3370	4605.99609	224.37221	72.0246
38	23.283	VV	0.2336	19.59741	1.10244	0.3064
39	23.514	VV	0.1249	9.39395	1.07900	0.1469
40	23.735	VV	0.1586	4.17714	3.72298e-1	0.0653

Totals : 6395.03614 491.68207

*** End of Report ***

Work continued from Page

④

Test - 20mav DMT- off U3P6W25

5 Took 20 μ , dried it down, dissolved
in 50 μ of DI H_2O , added 60 μ of
Enzyme mix. Incubated all

10 the sampler at ~~60°C~~ 37°C overnight

1/22/98

15 The Enzyme digests were seen
on C-18 reverse phase HPLC
using

20 Buffer A 0.1M TEAA 6.5 pH

— B 25% ACN in 0.1M TEAA

25 2- 25% B in 20 minutes

Method 25-2

50-2

30 ① No indication of transamination
in case of C was observed.

② The N+ peak was greatly reduced.
DEA can be used for decyanoethylation prior
to work up by NH_4OH .

Work continued to Page

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Work continued from Page

Synthesis of T(Gr- 20 mev
(LJP- 20 mev DmTr- ON) in a
24 ml Column.
diameter = 35 mm

0.2 molaw amudites

Gr- support 94 μ mol loading.

weight of Column =

134.9374

weight of Column + Support = 141.6613

weight of Support = 6.7 gm

Scale Synthesis = 630 μ mol

Weight after synthesis scale.

for 35 mm diameter linear flow
rate compared to 1ml Column

1ml = ~~0.5 cm~~ 5 mm 10 mm $35/10 = 3.5$

$3.2 = 4.9$

$\approx 12.5 \text{ mL/min}$

so for 1ml flow rate in 1ml Column
you need 12.5 ~~4.9~~ ml in 24 ml Column to
keep the same back pressure

Work continued from Page

The syn in the 24 mL Column
was taken for Method V3P6Wash
using 2.0 molar Diethylamine in
ACN dry.

Flow rate 1215 mL / minute
Time = 10 minutes

After the method was over, it was
washed with an extra 400 mL
of ACN from reagent pump.

V3P6Wash

Weight of the Synthesis after
V3P6Wa 20 145.76

difference = 10.8226 gms

0.937 AU/mL
10 mL Total

Deprotection

① Deprotection at R.T 0.1374 gm (3:30 PM 1/14/99)
② Deprotection at 60°C 0.1856 gm (3:30 PM 1/14/99)
10 mL Total Vol
10 mL = 1.2 AU / mL 1200 AU in 0.1856 gms
10.8226 will have = 69973.707 AU or

111 AU / μ mol

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Work continued from Page

Synthesis of LJP- 20 mer DMTr-ON
 in 6.3 mL column (Syn done for
 decyanoethylation prior to deprotection and
 cleavage)
 Weight of column = 63.8390

Weight of column + support = 65.5311
 1.6921 support

Scale = 159 μ mol scale

Gr- 94 μ mol loading.

Cap = Isobutyric anhydride 35% in
 ACN (This keeps the molarity at
 par with 20% AC_2O in ACN).

Weight after Synthesis and

V3P6Wa21

For Decyanoethylation: (2.0M Diethylamine in ACN)

Linear flow rate for 6.3 mL column =

For 1 mL Dia = 10 mm $20/10 = 2$ $2^2 = 4$
 — 6.3 Dia = 20 mm

4 mL/minute

for 10 minutes.

Weight after Decyanoethylation = Not much different

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C-20MER, DMTr-ON

BOOK NO.

Work continued from Page

Synthesis of C-20mer DMTr-ON
 using a 6.3 mL column, IBu anhy-
 Cap.

C-^{AZ} loading = 81 μ mol

Scale = 139.2 μ mol

wt of Column = 63.8462

+ support = 65.5649

1.7187

wt after Synthesis = 66.6079

2.7617 gms

The Synthesis was not washed
 in the column for decyanoethylation

amount of material for deprotection
 at 60°C for 16 hrs

0.198 gms in 3 mL NH₄OTf

Work up - 10 mL. 10A = 1.086 AU/mL = 1086 AU

15147.506 AU / 2.7617 gms

0.198

OR = 108.81 AU/ μ mole

REBINDERY PRODUCTIONS CHICAGO 60605 MADE IN USA

Work continued to Page

D.

DATE: 7.1

ED TO AND UNDERSTOOD BY

DATE

WITNESS

Bhavs

Work continued from Page

① C-20mer DMTr-ON
V3P6Wa26

1mL Column filled with C-20mer T-ON
on support (IBu capped) washed with
2.0 molar DEA in ACN @ 1mL/minute
for 10 minutes.

wt for desprotection at 60°C for 16h
0.165gms

after work up total Volume = 10 mL

$$10 \mu = 0.936 \text{ AU/mL} = 936 \text{ AU/10mL}$$

② C-20mer DMTr-ON
V3P6Wa27

1mL Column filled with C-20mer T-ON
on support (IBu capped) washed with
2.0 molar DEA in ACN @ 1mL/minute for

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WITNESS

DATE

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20 minutes

wt. for deprotection =
at 60°C for 16hrs 0.163 gms

after work up total Volume = 10 mL

$$10\lambda = 0.944 \text{ AU/mL} = 944 \text{ AU/10 mL}$$

(3) C-20 meq DmTr-ON

U3P6Wa 28

1 mL Column filled with C-20 meq T-ON
on support (IBu capped) washed with
2.0 mL of DEA in ACN @ 1 mL/minute for
30 minutes.

wt for deprotection at 60°C for 16hrs
= 0.162 gms

after work up total Volume = 10 mL

$$10\lambda = 0.924 \text{ or } 924 \text{ AU/10 mL}$$

All samples were taken for
analytical chem.

Work continued to Page

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Work continued from Page

Test- 20 Syn DmT- off

01159901 PHF, regular cap.

① amount for deprotection
at 60°C 16 hrs = 0.179 gms.

work up 10 mL

$$10\lambda = 1.359 \text{ AU} \quad \text{or} \quad 1359 \text{ AU/10mL}$$

② 01159901 PHF V3P6Wa25

Washed in 1mL Column with 2.0molar
DEA in ACN @ 1mL/minute for 10 minutes

wt for deprotection = 0.167 gms

work up 10 mL

$$10\lambda = 1.247 \text{ AU/mL} \quad 1247 \text{ AU in 10mL}$$

Samples taken for analytical Chrom

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Work continued from Page

Enzyme digestion Expts.

①

C-20 men DmTr- ON

Took 30 λ , ^{N(3AU)} dried at low heat
of NH_4OH /EtOH extract.

added 60 λ of Enzyme mix (made
from previous batch) to a 50 λ of
the dried material in H_2O .
Incubated at 37°C overnight.

②

C-20 men DmTr- ON VBP6Wax8

Took 30 λ , ^{N(3AU)} dried down at low heat
dissolved in 50 λ of H_2O and added
60 λ of Enzyme mix

③

Test-20 men DmTr- off

Took 20 λ . N(3AU) dried down at low
heat, dissolved in 50 λ of H_2O and added
60 λ of Enzyme mix

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Anilabhan

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